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Molecular characterization of *Gymnema sylvestre* using RAPD-PCR

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ABSTRACT

Herbal medicine is playing a major role in the control of many diseases, especially in developing countries like India. The conservation and utilization of these plants has attracted global attention. Diabetes mellitus is a endocrine syndrome which is characterized by insulin insufficiency or inefficiency. Besides many oral hypoglycemic drugs, there are certain anti-diabetic plants which can be used for the treatment of diabetes mellitus. *Gymnema sylvestre* is one of those anti-diabetic plants. To improve the cultivar of this *Gymnema sylvestre*, identification of their molecular markers should be done. RAPD-PCR can generate these molecular markers. The main objective of this study is to demonstrate the polymorphism that exists between five different ecotypes of *Gymnema sylvestre.* The RAPD-PCR was done with four primers. All the primers gave satisfactory amplification with maximum number of bands except one. Some bands were monomorphic and some were polymorphic. Thus the result reveals the presence of genetic diversity among five ecotypes of *G.sylvestre*.

Keywords: *Gymnema sylvestre,* PCR, RAPD, DNA, anti diabetic plant.

INTRODUCTION

A Plant, which provides health-promoting characteristics, temporary relief or symptomatic problems or has curative properties, is generally called as medicinal plants. The medicinal plants are in great demand in tradition system of medicine i.e. ayurveda, siddha, unani and rig-veda, tibb as well as folklore prescriptions.

About 80% of four billion people rely on traditional medicines due to high cost and lack of availability of required medicines. Out of 250,000 higher plants, more than 80,000 are having medicinal value and India occupies a unique position among world biodiversity center. It is estimated that 13,000 medicinal plant species are being used for the production of drugs. In India, almost 45,000 plant species are growing naturally or being cultivated. Herbal medicine is playing a major role in the control and cure of many diseases and about 75%-80% of the world's population is utilizing these medicines, especially in developing countries due to lesser side effects.

Medicinal and Aromatic plants (MAPS) utilization and conservation has attracted global attention [1]. Several of these MAPS contained exceptionally high amounts of polysaccharides, polyphenols, tannins, hydrocolloids (sugars and carragenans) and other secondary metabolites such as alkaloids, flavanoids, phenols, terpenes and quinines which would interfere with the DNA isolation procedures [2].

Gymnema sylvestre, a family of Asclepidaceae, is a plant native to the tropical forests of India, and it has been used to treat a number of conditions. It is best known for its apparent ability to lower blood sugar levels. [3, 4,5,6,7,8,9]. Medicinally active parts of the plant are the leaves and roots. The active principle of this plant is an organic acid called gymnemic acid [10, 11]. This gymnemic acid suppresses the sweet taste not only of sucrose, but also the sweetness effect of sodium saccharin, cyclamate, glycine, D-alanine, D-tryptophan, D-leucine, beryllium chloride and lead acetate but not that of chloroform. [12,13,14,7,8,9]. Scientists from India find that the ethanolic extract of *gymnema Sylvester* leaves demonstrated antimicrobial activity against Bacillus pumilis, B. subtilis, Pseudomonas aeruginosa and Staphylococcus aureus and inactive against proteus vulgaris and Escherichia coli. [15]. Besides these properties gymnema also lower the serum cholesterol and triglyceride levels. [16].

The RAPD technique provides a convenient and rapid assessment of the difference in the genetic composition of the related individuals and has been employed in a large number of plants for the determination and assessment of genetic diversity. [17]. RAPD markers have been used for generating genetic linkage maps. [18,19,20] genotype fingerprinting [21], analyzing populations and pedigree [22], predicting phylogenies [23], studying population dynamics [24] and identifying clones [25].

RAPD reveals better polymorphism patterns and their primers used are universal. Laity fall (2003) [26] used RAPD to evaluate the genetic variations among cowpea varieties for the highest nitrogen fixing capacity[27] have stated that molecular markers are

used to identify cultivars with much greater reliability than the morphological traits which are governed by complex genetic interactions.

Similar studies on P. hexandrum [28], lotus [29], sweet potato [30] and Andrographis paniculata [31] suggest that RAPD is more appropriate for analysis of genetic variability in closely related genotypes.

The main objective of the present study is to demonstrate the genetic diversity among five ecotypes of *Gymnema sylvestre* using RAPD analysis and we hope that this present study may throw light on better improvement in the development of a new cultivar of *Gymnema sylvestre*, which could be effectively used for treating diabetes.

MATERIALS AND METHODS

***Collection of plant material & Isolation of plant genomic DNA* :**

Different ecotypes of gymnema plants were as shown in table 1. After acclimatization, 1g of young leaves was harvested fresh for DNA isolation.Total genomic DNA was extracted from the young leaves of Gymnema sylvestre using CTAB method [2] in which the leaves were subjected to pretreatment with CTAB - DNA extraction buffer at 60ºC. Then the DNA was extracted using chloroform - isoamylalcohol mixture followed by isopropanol precipitation. The extracted DNA was treated with phenol and chloroform mixture followed by Rnase treatment to remove the impurities like RNA and protein

RAPD Amplification

PCR amplifications were performed in a 0.2 ml tube containing 15 mM $MgCl_2$, 300ng of each primer, 200 mM of each dNTP, 1 U of lyophilized Taq (Amersham Pharmacia Biotech) and 100 ng of genomic DNA in a final volume of 25ml. Four primers (Genosphere Biotechnologies) were used in this study. The reaction were placed in a PTC-100 thermocycler (MJ Research Inc.) programmed for predenaturating step of 5 min at 94°C followed by 40 cycles of 1 min 94°C, 1 min at 36°C, 2 min at 72°C and a final extension of 5 min at 72°C. (Table 2 shows the primer sequence)

Agarose gel electrophoresis

The amplified products were analyzed on 1.5% agarose gel. The gels were stained for 30 min with 1mg/l ethidium bromide and photographed with 667 Polaroid films under UV light & scored for the presence / absence of polymorphic bands.

RESULTS

A preliminary study was carried out using only four primers namely, OPL 01, OPL 02, OPL 06, & OPL 07. The total number of amplified bands, number of polymorphic bands; number of exclusive bands obtained by RAPD is shown in Fig 1, 2, 3, and 4.

Except OPL 01 primer all the primers gave satisfactory amplification with maximum number of bands. Five amplicons were observed with the primer OPL 01 and they range from 500bp to 1000bp. All the bands were found to be monomorphic.

The primer OPL 02 resulted with a total number of 30 amplicons. Ten bands were found to be polymorphic and three were specific to the ecotypes I, ecotype III and ecotype V (Fig.2) and the amplicons ranged from 500 bp to 4500 bp. A total number of 19 bands were amplified with the primer OPL 06 with the fragment sizes ranging from approximately 263 bp to 3278 bp. Eight out of these 19 bands were polymorphic.

21 amplicons were observed with the primer OPL 07 and their range from 500 bp to 3500 bp. All the bands were monomorphic except one, which is specific for the ecotype II (Fig.4).

DISCUSSION

Molecular characterization of the plant is required in order to distinguish among varieties of a specific plan [32]. Study of inter and intraspecific variation at the molecular level provides an efficient tool for taxonomic and evolutionary studies and for devising strategies to protect genetic diversity of species. Genetic variability also can be exploited to select useful genotypes that could be utilized as cultivars to avoid batch-to-batch variation [28].

The use of random primers in a PCR is a powerful tool that reveals extensive DNA polymorphism, and it has become valuable in genetic analyses. Since RAPD - PCR does not require prior sequence information and an arbitrarily chosen short primer is used at lower annealing temperature than routine PCR to amplify one or multiple DNA segments from genomic DNA, a large number of polymorphic DNA markers may be easily generated. Different ecotypes of *Gymnema sylvestre* were collected from various places of Andhra Pradesh, kerala, pune and orissa.

RAPD analysis was carried on all these five ecotypes and it was found that there was a difference in the genetic make up of these five different ecotypes. The genetic diversity among the five ecotypes of gymnema provides us to develop molecular markers, which may improve the cultivars of *Gymnema sylvestre. Since gymnema* was a typical medical herb which has

antidiabetic , antimicrobial, cholesterol reducing property, the present study may throw light on the production of a new cultivar of *Gymnema sylvestre*, which could be effectively used for treating diseases.

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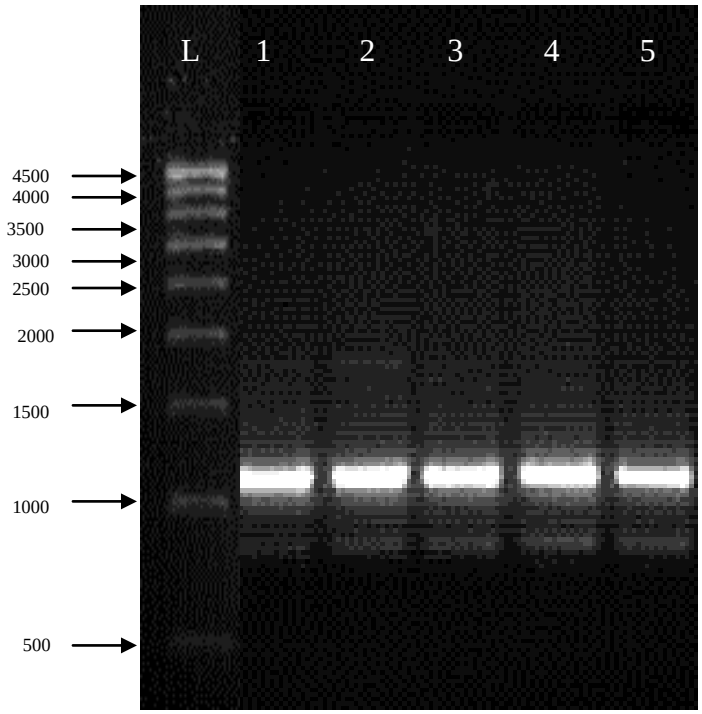

L - 5000 Bp Ladder: 1 -Ecotype I : 2 - Ecotype Ii: 3 - Ecotype Iii :4 - Ecotype Iv :5 - Ecotype V

Primer OPL-01 gave the monomorphic bands and the amplicons ranged from 500 to 1000 bp

Fig.1 RAPD Profiles Of Different Ecotypes Of Gymnema sylvestre With OPL-01 Primer

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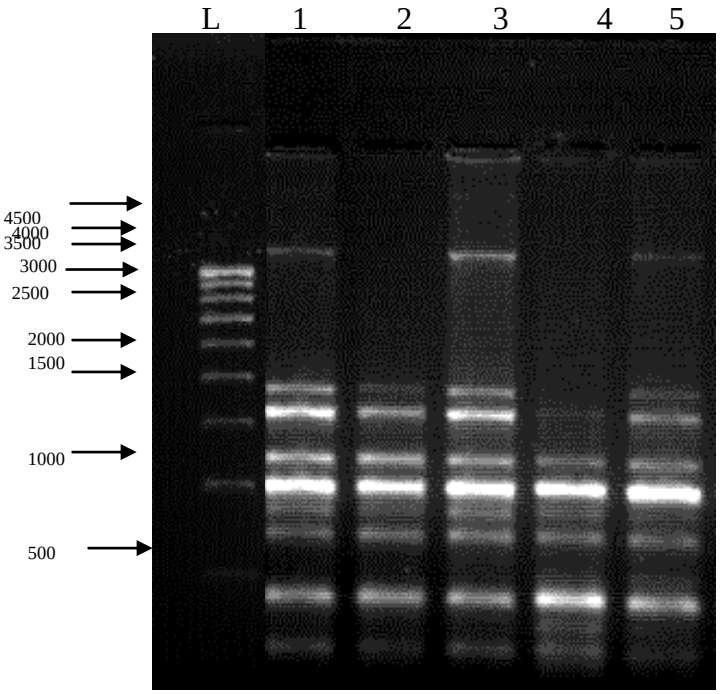
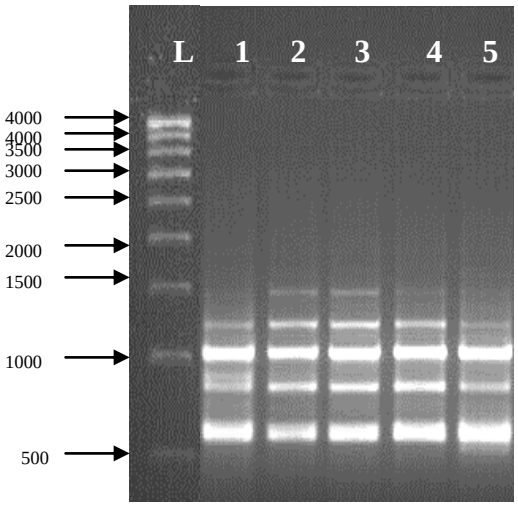


L - 5000 BP LADDER: 1 - Ecotype I: 2 - Ecotype II: 3 - Ecotype III :4 - Ecotype IV :5 - Ecotype V

Primer OPL_02 gave maximum no of bands. The amplicons ranged from 500 bp to 4500bp.

Fig.2 RAPD Profiles Of Different Ecotypes of Gymnema sylvestre With OPL-02 primer



L - 5000 bp Ladder: 1 - Ecotype I: 2 -Ecotype II: 3 - Ecotype III: 4 - Ecotype IV: 5 - Ecotype V

Primer OPL_06 gave maximum no of bands. The amplicons ranged from 500bp to 2500bp.

Fig.3 RAPD Profiles Of Different Ecotypes of Gymnema sylvestre With OPL-06 Primer

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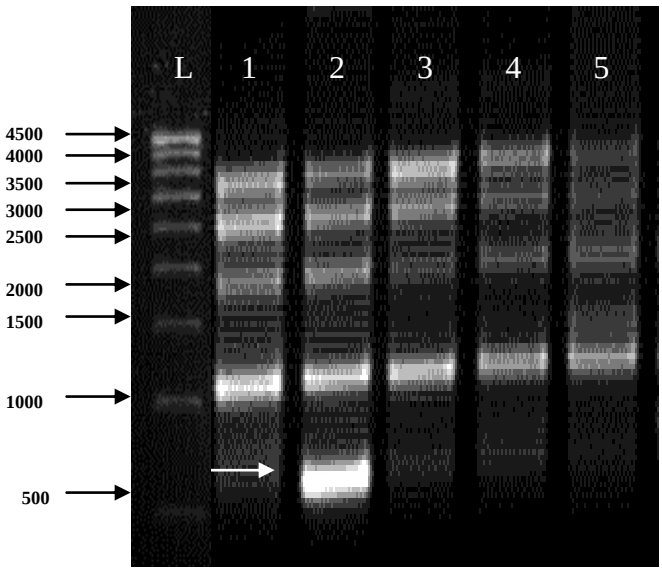

L - 5000 BP LADDER: 1 - Ecotype I: 2 -Ecotype II: 3 - Ecotype III: 4 - Ecotype IV: 5 - Ecotype V

Primer OPL-07 gave the all monomorphic bands except one i.e., specific to the ecotype 2 and the amplicons ranged from 500 bp to 3500 bp

Fig.4. RAPD Profiles Of Different Ecotypes of Gymnema sylvestre With OPL-07 primer

Table 1. Summarizing the different ecotypes of *Gymnema sylvestre*

Eco Type	Place
I	Forest Research centre, Mulugu, A.P
II	Tropical Botanical Garden Research centre, pallad, kerala
III	Forest Research centre, Rajahmundry, A.P
IV	Forest Research centre, pune Westernghats.
V	Forest Research centre, Bhubaneswar, orissa.

Table 2. Sequences of primers used in RAPD PCR

OPL-01	5'GGC ATG ACC T3'
OPL-02	5'GGT GGG CGT CAA3'
OPL-06	5'GAG GGA AGA G3'
OPL-07	5'GAG GGA AGA G3'